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14. ABSTRACT

We have demonstrated that (1) alcohol-naïve rats exhibiting high acoustic startle response (associated with increased anxiety-like behavior) develop increased subsequent alcohol intake and preference which are correlated with acoustic startle response determined before initial alcohol access, providing a prospective index of vulnerability to developing alcohol abuse, as well as insights into mechanism; (2) suppression of noradrenergic signaling decreases alcohol drinking in rats with a history of traumatic stress, but not in rats without this stress history (informing clinical studies in which subjects exhibit variable responses to prazosin); (3) this treatment also suppresses alcohol drinking by rats with history of compulsive-like alcohol drinking, but increases alcohol drinking by rats that do not (these results inform clinical studies in which subjects have been reported to exhibit opposite responses that are dependent on family history of compulsive alcohol drinking; (4) suppression of noradrenergic signaling at the time of traumatic stress decreases acquisition of increased voluntary alcohol drinking long after the stress, which provides a new model for preventive treatment. Accomplishment 1 has been published, 2-4 are in preparation for publication. All remaining proposed studies are currently in progress as planned, with no changes in scope, although with some delays due to personnel changes and due to the need for some methodology refinements.

15. SUBJECT TERMS

PTSD, alcohol, ethanol, prazosin, noradrenergic, startle, anxiety, stress, pharmacotherapy, prevention, rat, abuse

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1. **INTRODUCTION:** Studies from our research group demonstrating that the well-characterized, safe, well-tolerated and FDA-approved α1-adrenergic receptor antagonist (AR), prazosin, is effective not only in treating combat post-traumatic stress disorder (PTSD) symptoms but in decreasing alcohol drinking in both human and rat studies provide much-needed breakthroughs in the development of effective pharmacotherapies for alcohol use disorders as well as for PTSD. However, much work remains to determine conditions in which this treatment to reduce noradrenergic hyperactivation will be effective, characteristics of individuals who are most likely to respond, and underlying mechanisms providing bases for additional treatments. Our immediate objective is to identify key variables in rat models that will inform and complement human studies, providing a powerful translational approach for most efficiently and rapidly developing and implementing effective new pharmacotherapies for alcohol use disorders and co-morbid PTSD.

2. **KEYWORDS:** alcohol, ethanol, PTSD, prazosin, noradrenergic, startle, anxiety, stress, pharmacotherapy, prevention, rat, abuse

3. **OVERALL PROJECT SUMMARY:** There are no significant changes in the project goals or studies planned.
    - **CURRENT OBJECTIVES** There are 3 objectives of this project, which have remained unchanged. To be consistent with the organization and section headings of the current SOW, these 3 major objectives continue to be presented here as SPECIFIC AIMS 1-3, although each of these specific aims now also are identified (in parentheses) as Objective 1, 2 or 3, for further clarity. Also consistent with the organization of the current SOW, Tasks 1-6 are discussed within these three SPECIFIC AIMS (Objectives), identified by bold text.
        - **SPECIFIC AIM 1 (Objective 1): Determine relationship of hyperexcitability, anxiety and α1-adrenergic receptor-mediated signaling to excessive voluntary alcohol drinking**, *providing information from rat models that will likely reveal especially promising bases for:*

a) Prospectively identifying subsets of individuals who are highly vulnerable to developing alcohol use disorders (AUDs). **(TASK 1)**

STATUS: We have demonstrated that alcohol-naïve rats exhibiting high acoustic startle response (which is associated with increased anxiety-like behavior) develop increased subsequent alcohol intake and alcohol preference in an intermittent alcohol access (IAA) paradigm. These results are consistent with the central hypothesis for all other studies in this research project, i.e., that hyper-responsiveness characteristic of PTSD, alcohol withdrawal/abstinence, and increased noradrenergic activation contributes to – or at least is associated with – development of increased alcohol drinking. This work was completed in year 1 and

described in detail in the Year 1 progress report. The published paper is included in the appendix (Rasmussen and Kincaid, 2015).

b) predicting who is most likely to respond to prazosin with decreased alcohol drinking. **(This work was not assigned a separate specific TASK # in the SOW, but it is a component of several of the proposed experiments. To facilitate discussion, we include it here as TASK 1b**).

STATUS: Although our initial work suggests that high acoustic startle and increased anxiety-like behavior is associated with increased suppression of alcohol drinking in response to prazosin, as hypothesized, we are investigating responses in all alcohol access and PTSD-like conditions of this overall investigation, as planned. Consequently, resolution of this issue will not be finalized until all experiments are completed. As described in the original proposal, prazosin is being administered prior to voluntary alcohol drinking in rats that have been previously characterized for acoustic startle and anxiety-like behaviors in the differing experimental models used in these studies; we continue to evaluate whether prazosin treatment disproportionately decreases alcohol drinking in those rats with pre-existing or PTSD-induced high acoustic startle and high anxiety-like behavior. Nonetheless, an early result is already of great interest and likely clinical relevance. In an initial trial with the rat PTSD model used throughout these investigations (and described in detail in the proposal), 51 young male Wistar rats received either 10 seconds of inescapable footshock (traumatic shock; TS) or no shock (NS) followed by 4 weekly contextual reminders (R) of the TS or NS (but no further application of TS or NS). The rats were then provided 4 weeks of intermittent alcohol access (IAA, 24 h/day free choice between 20% alcohol *vs* water on 3 non-consecutive days/week) to establish stable elevated levels of voluntary episodic alcohol drinking. Alcohol access was then further restricted to 1 hour on each IAA day for the next 4 weeks. On 1 day of each week, each rat received an intra-peritoneal (IP) injection of either vehicle alone (VEH), the alpha-1 adrenergic receptor antagonist, prazosin (PRZ, 1.5 mg/kg), the beta-adrenergic receptor antagonist, propranolol (PROP, 5 mg/kg), or the combination of PRZ+PROP (1.5 mg/kg, 5 mg/kg) at 30 min before the 1 hour alcohol access period, with each rat receiving each of the 4 treatments in counterbalanced order over the 4 weeks. In the rats that had not received TS ("Non-Stress" in the figure) there was not a significant change in alcohol intake in response to either drug alone or to the combination of drugs (**Fig. 1**), even though the doses of each drug were the same as we have previously demonstrated to consistently decrease alcohol drinking in selectively-bred alcohol-preferring (P) rats derived from the Wistar strain. In contrast, rats that had received a single application of TS at least 8 weeks before testing ("Traumatic Stress" in the figure) exhibited consistently decreased acute alcohol intake in response to prazosin treatment, and this suppression was enhanced by combining the prasozin treatment with propranolol

treatment (this enhanced suppression of alcohol drinking by addition of propranolol to prazosin treatment is consistent with our previous results in P rats). These results suggest that, in normal outbred rats voluntarily drinking alcohol in an IAA model, the response to reduction of noradrenergic activity by prazosin or prazosin+propranolol is dependent on history of previous stress.

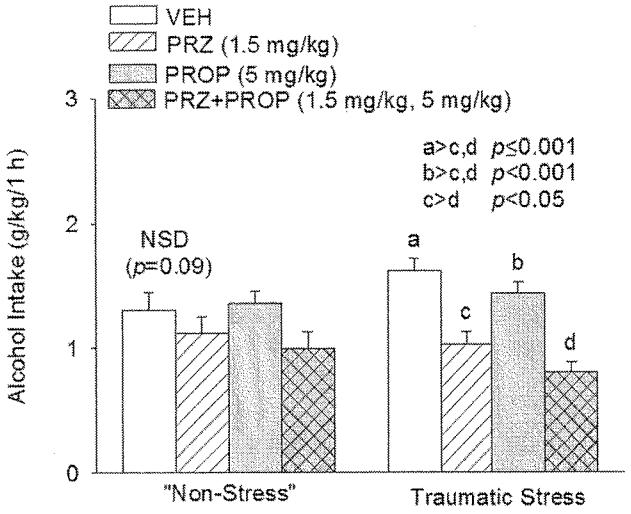

FIG. 1

Due to the high potential clinical significance of these results, we have now repeated this entire experiment with another set of rats, again starting with the PTSD model followed by 4 weeks intermittent alcohol access and then this same acute drug testing. The results are shown in **Fig. 2**. In this repeat experiment, prazosin or prazosin+propranolol again each reduced al cohol drinking in rats with a history of single traumatic stress 13 weeks earlier but – again - not in rats that had received non-stress treatment. Together, these two complementary experiments now provide consistent evidence that a single 10 second traumatic stress with occasional brief contextual reminders produced conditions in which prazosin or prazosin+propranolol more effectively reduced alcohol drinking long after an acutely traumatic experience.

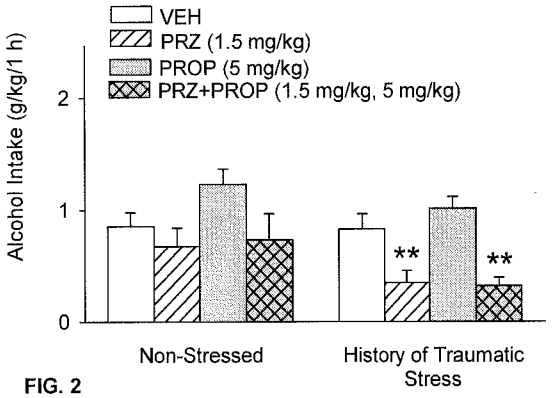

FIG. 2

Our finding that effectiveness of prazosin or prazosin+propranolol in suppressing alcohol drinking is dependent on preceding history of traumatic stress will likely have great impact in informing further clinical trials and applications of prazosin treatments, since clinical responses to prazosin treatment have been inconsistent. Further characterization of relationships of this response to rat PTSD-related behavioral predictive indices will increase this utility and impact. In addition, this rat model may provide an especially effective preclinical model for further resolving the

brain, neuroendocrine and behavioral mechanisms mediating this disparity in responses.

We recently presented this work at the 2017 Annual Meeting of the Research Society on Alcoholism, in a symposium entitled "The Neglected Catecholamine: Noradrenergic Mechanisms in Regulation of Alcohol-Related Behaviors". Dr. Rasmussen was Co-Organizer of this symposium. His research presentation, entitled "Variability in alcohol drinking responses to suppression of noradrenergic signaling in outbred rats" also included additional new results from a study employing long-term [IAA vs continuous access] model of compulsive alcohol drinking that is discussed later in this report.

c) preventing initial acquisition of AUDs in prospectively-identified vulnerable individuals. **(TASK 2)**

STATUS: The central hypothesis of this experiment is that rats exhibiting high acoustic startle before the initiation of IAA will subsequently exhibit high IAA alcohol intake (as we have demonstrated in Task 1), and that continuous treatment to suppress noradrenergic signaling before and throughout IAA will prevent this acquisition of high alcohol drinking. This experiment thus requires continuous treatment of the rats during introduction of IAA. As discussed in detail in the previous (Year 3) report, we had repeated problems with several approaches to providing this continuous treatment. We had originally proposed to accomplish this with intraperitoneal administrations 3 times each day, which proved to be highly stressful within only a few days. We then instead conducted an entire study using implantable osmotic minipumps to maintain prolonged (4 week) constant administration (with prior approval at the time of the first annual report). Figures presenting results were included in the previous progress report (Year 3). In short, there were two major problems. The first was that osmotic pumps delivering only vehicle suppressed alcohol intake compared to untreated rats, suggesting that either stress associated with the implanted pump or perhaps the administration of vehicle alone suppressed alcohol drinking. The second was that prazosin or [prazosin+propranolol] were ineffective in consistently altering the alcohol drinking. This lack of response was eventually determined to be due to the fact that the prazosin had precipitated out of solution within the prazosin and [prazosin+propranolol] pumps, clogging the orifice through which the drug is delivered. The process of confirming this problem source was extensively discussed in the Year 3 progress report. Consequently, as proposed in the Year 3 report, we have now repeated this study using an oral route of administration. In order to circumvent the known problem of prazosin short metabolic half-life that requires multiple daily treatments over the entire 4 week treatment period, we proposed in the year 3 report to instead use the drug doxazosin, an analog of prazosin which is functionally essentially identical - with the same alpha-1 adrenergic receptor antagonist specificity - but which has a much longer half-life that allows once/daily administration in clinical applications. We

have already reported that doxazosin suppresses alcohol drinking in rats comparably to prazosin, and doxazosin has recently been reported (by a group including my colleague and consultant, Dr. Murray Raskind) to suppress clinical symptoms of PTSD similarly to prazosin. To allow investigation of multiple doxazosin dosing (as originally proposed for prazosin), we proposed (in the Year 3 report) to investigate only VEH vs doxazosin treatments. So, although the proposed study was completed, we have now adjusted the model to eliminate problems that we had encountered and to repeat the study again, this time with doxazosin administered in pieces of flavored gelatin which the rats find to be palatable, even when containing dissolved doxazosin. . The results with the first cohort of animals are now shown in **Figs. 3 - 5**. The treatment groups are Vehicle (flavored gelatin alone), 1 Doxazosin (5 mg/kg doxazosin administered once, at start of dark period), and 2 Doxazosin (5 mg/kg doxazosin administered twice; once at the s tart of the dark period and again near the end of the dark period). As illustrated in **Fig. 3**, administration of doxazosin 1x/day produced a small reduction in the development of IAA alcohol drinking, and 2X/day produced a marked suppression of this initial development of increased alcohol drinking. **Fig. 4** illustrates that daily treatment with doxazosin likewise suppressed development of increased alcohol preference (relative to water). **Fig. 5** illustrates that 1x/day doxazosin appears to be potentially most effective in suppressing development of increased IAA alcohol drinking in the subset of rats that had the lowest acoustic startle response (measured here as Vmax) characterized before the start of alcohol access, although variance in the results is hi gh and the sample size is inadequate for a robust test. Consequently we will repeat this trial with an additional cohort of rats to more effectively resolve validity of pre-IAA indices in predicting response to doxazosin.

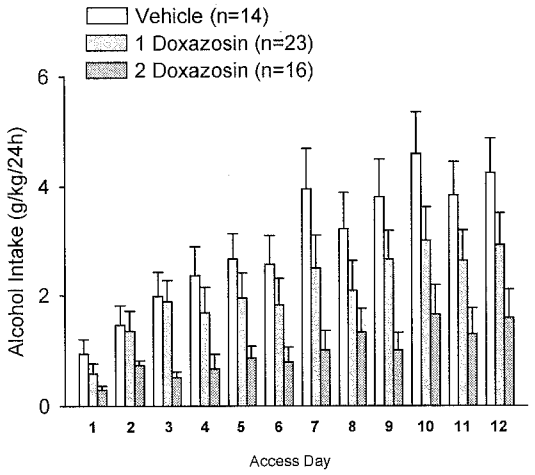

FIG. 3

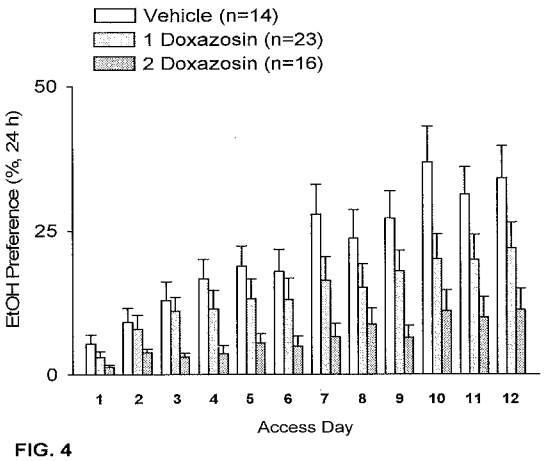

FIG. 4

Since we have now established a reliable model and methodology, completion of this investigation should proceed smoothly.

d) predicting who is most vulnerable to progression from voluntary to compulsive alcohol drinking. **(TASK 3)**

STATUS: This prolonged (28 week) study evaluates the hypothesis that prazosin will suppress high alcohol drinking even in rats that had exhibited high acoustic startle and associated anxiety-like behavior before IAA and which subsequently develop compulsive alcohol drinking (i.e., alcohol drinking that is maintained even after a distasteful adulterant – e.g., quinine - is added to the alcohol). The basic animal work for this part of the project has now been completed. As we recently reported at the 2017 Annual Meeting of the Research Society on Alcoholism symposium organized by the PI, entitled "The Neglected Catecholamine: Noradrenergic Mechanisms in Regulation of Alcohol-Related Behaviors", rats that received alcohol in the IAA model for 24 weeks developed markedly elevated levels of alcohol drinking that was compulsive-like (i.e., it was resistant to adulteration of the alcohol with bitter tasting quinine) whereas rats that received continuous access to alcohol did not develop elevated levels of consumption and the consumption was not compulsive-like. Furthermore, the compulsive-like alcohol drinking of the IAA rats was suppressed by doxazosin treatment, whereas non-compulsive alcohol drinking in the rats receiving continuous alcohol access was conversely increased by doxazosin treatment (**Fig. 6**).

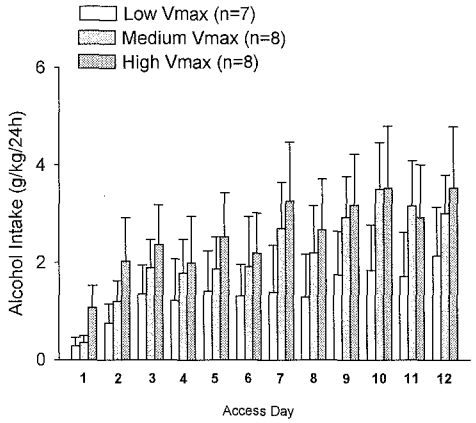

FIG. 5

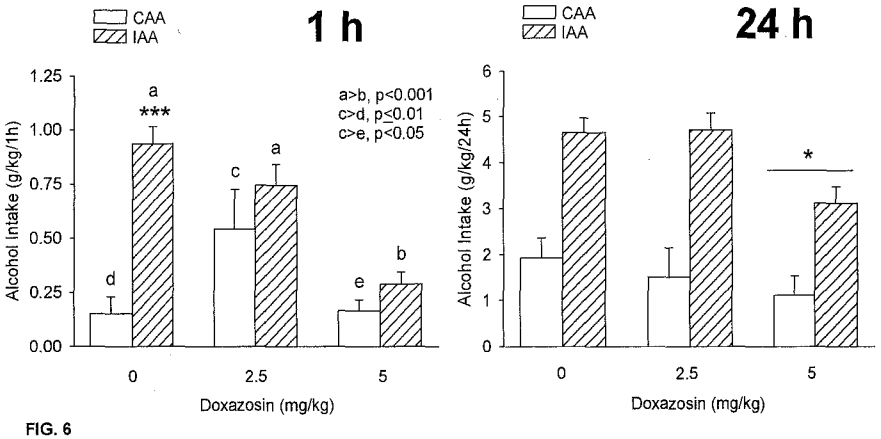

FIG. 6

These results were surprising, but consistent with results of a recent clinical study in the laboratory of Lorenzo Leggio, MD PhD (which were

also presented in our symposium at the 2017 Research Society on Alcoholism meeting) - i.e., Dr Leggio demonstrated that alcoholic subjects who are family history (+) for alcoholism continue to drink high levels of alcohol even in the clinical trial setti ng (i.e., they exhibit evidence of compulsive drinking) and also respond to doxazosin with decreased alcohol drinking, whereas alcoholic subjects who are family history (-) exhibit markedly decreased alcohol drinking in the clinical trial setting (i.e, the clinical setting produces a large placebo effect consistent with non-compulsive drinking) but doxazosin paradoxically increases this alcohol drinking. The surprising results with our rat model thus are remarkably consistent with clinical evaluations with alcoholic subjects. Furthermore, our results in this study complemented results in our study described above in Task 1b (and also presented in this same 2017 symposium) demonstrating that although prazosin administration to rats that had received prior traumatic stress produced decreased alcohol drinking, prazosin administration to rats that had not received prior traumatic stress did not. As highlighted by the Discussant/Question Moderator for the symposium, Dr. Raye Litten of the National Institute for Alcohol Abuse and Alcoholism (NIAAA), these results strongly suggest that a subset of subjects who are predisposed to develop compulsive alcohol drinking due to genetics, prolonged severe alcohol abuse, and/or traumatic stress history may be the subset of otherwise difficult-to-treat subjects most appropriate for treatment to reduce noradrenergic signaling with prazosin or doxazosin.

We are currently further analyzing whether initial acoustic startle response and behavioral characterization can predict the subsequent development of compulsive-like drinking and also the response to prazosin or doxazosin. We do not anticipate that any additional animal work will be necessary, although extensive (and time intensive) analyses are still required.

SPECIFIC AIM 2 (Objective 2): Evaluate PTSD/alcohol interactions, *providing information from rat models that will likely reveal especially promising bases for:*

a) determining cause-effect between AUDs and vulnerability to developing PTSD. **(TASK 4)**

STATUS: This work compares production of a PTSD-like behavioral and acoustic startle profile in rats with *vs* without a previous recent history of alcohol liquid diet-induced excessive prolonged alcohol intake. This work likewise is nearly complete (see discussions of CHANGES, PROBLEMS, DELAYS AND ACTIONS TO RESOLVE THEM in the Year 2 and 3 Annual Reports). We have already published a demonstration that symptoms in our rat PTSD model predict subsequent significantly increased development of excessive alcohol drinking, and we have further confirmed in a study reported at the 2016 Annual Meeting of the Society for Research on Alcoholism that PTSD in our rat model increased alcohol

drinking. We are now conducting data analyses for the entire proposed investigation. A second part of this investigation is to determine whether a history of chronic high alcohol consumption and withdrawals increases vulnerability to develop PTSD following a traumatic stress (TS) during abstinence. In the proposed completed experiment addressing this issue, provision of liquid diet containing alcohol for 3 cycles of 5 days alcohol, interrupted by 2 days of isocaloric control diet following cycles 1 and 2 (compared to 19 days of continuous control diet) produced increased acoustic startle after more than one month of imposed abstinence (**Fig. 7**; EtOH+NS), consistent with our previous work demonstrating that chronic alcohol liquid diet-induced dependence produced increased acoustic startle after 1 month of imposed abstinence, together with other PTSD-like neuroendocrine and behavioral symptoms. A single 10 sec TS after the 19 days of control diet (i.e., no alcohol exposure) followed by 4 weekly reminders of the stress (i.e., our rPTSD model) likewise increased acoustic startle (**Fig. 7**; PF+TS) consistent with our previous demonstration that this rPTSD model produces increased acoustic startle. However, when 3 cycles of 5 days of alcohol consumption were followed by traumatic stress applied 5 hours after removal of the alcohol diet followed by 4 weekly reminders, acoustic startle response was not increased (compared to rats receiving control diet without TS) (**Fig. 7**; EtOH+TS). This unexpected result led us to consider that perhaps rats receiving the alcohol liquid diet were still experiencing the pharmacologic effects of the alcohol even 5 hours after removal of the alcohol-containing diet, presumably due to slow stomach emptying of the high fat and high sugar liquid diet (resulting in relatively sustained delivery of alcohol) and slow clearance of the high dose of alcohol. If so, then the paradoxical lack of response to TS may have been due to sustained sedating and anxiolytic alcohol actions at the time of the TS, so that the TS was relatively non-traumatic. Consequently, we found it necessary to conduct another (unplanned) experiment, this time presenting the single TS during imposed abstinence one week after termination of the chronic alcohol consumption, rather than the 5 hours used in the study our protocol was modeled after, at a time that that we have confirmed plasma alcohol to have been completely cleared. In this new study with much delayed administration of the TS *vs* NS, ASR tested after 4 weekly reminders again was increased in the pair-fed control rats that had received TS

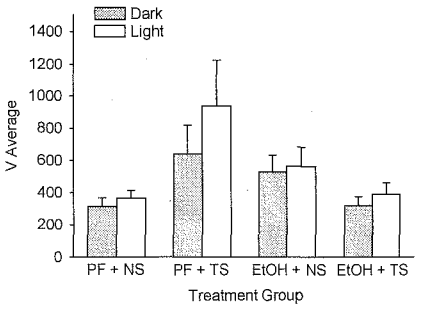

FIG. 7

(relative to pair-fed control rats that had received NS) but, surprisingly, chronic consumption of alcohol liquid diet that was terminated one week before the TS again appeared to block the subsequent expected increase in ASR, as illustrated by the ASR during brig ht light exposure in **Fig. 8**.

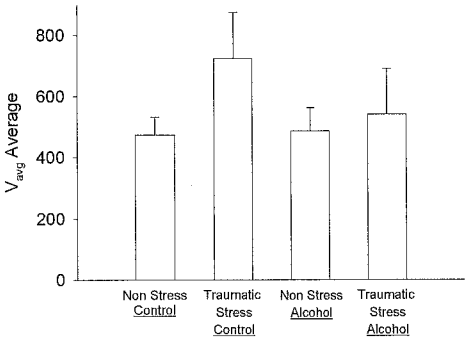

FIG. 8

So, although this experiment has now been conducted twice, and the experiments have provided similar results under the two different conditions of alcohol diet followed by TS, the interpretation is unclear. One possibility is that the liquid diet model may not have produced sufficiently robust alcohol dependence coupled with increased anxiety like behavior prior to the TS. We now plan to again repeat the study, maintaining the liquid diet treatment longer, with intervals of alcohol deprivation to test withdrawal behavior, and only administer the TS *vs* NS treatment when robust dependence has been confirmed.

b) predicting who, among individuals with PTSD, is especially vulnerable to developing AUDs **(TASK 5)**.

STATUS: This work addresses whether a rat PTSD-like behavioral and acoustic startle profile predicts subsequent acquisition of increased IAA alcohol intake. This work corresponds to **Task 5**. As discussed in the years 2 Annual Report, this work is being conducted in parallel with Task 6 (discussed below). These studies are being done in parallel because the methods and time schedules are compatible, with the major difference being that the study addressing Task 6 also incorporates pharmacologic treatment to decrease noradrenergic signaling at the time of traumatic stress. This is work is approximately 75% complete.

c) predicting who, among individuals with PTSD, is likely to respond to prazosin with decreased alcohol drinking.

STATUS: This work addresses whether a rat PTSD-like behavioral and acoustic startle profile predicts subsequent effectiveness of prazosin treatment in suppressing IAA alcohol intake. **As discussed in the years 2 and 3 Annual Reports, this work was not identified as a separate specific Task, but it is a component of several of the proposed experiments which were not all originally projected to be completed until the end of the overall project**. As discussed in the years 2 and 3 Annual Reports, this work was not scheduled as a separate specific task with a single proposed completion date, but it is a component of several of the proposed experiments which were not all originally projected to be completed until the end of the overall project. This work, which includes extensive analyses from several of the experiments, will still not be

completed until the completion of the overall; the necessary work is now approximately 90% complete. Note that - as discussed in Task 1b - alcohol drinking, which commonly is increased in individuals with PTSD as well as in our rat model of PTSD, was acutely suppressed by prazosin or prazosin+propranolol treatment in rats that had previously received traumatic stress and developed other rat PTSD symptoms, but not in rats that had not received traumatic stress 2 months earlier (presented these results as part of a 2017 Annual Meeting of the Research Society on Alcoholism symposium organized by the PI, entitled "The Neglected Catecholamine: Noradrenergic Mechanisms in Regulation of Alcohol-Related Behaviors"), and that – also discussed in Task 1b - we are in the process of characterizing this response in the context of other rat PTSD symptom characterizations which we have determined - due to the greater variability in expression of rat PTSD-like parameters - to require one additional cohort of subjects (rats) to increase subject numbers (n's) to allow adequate resolution for these additional characterizations, which is now in process.

- **SPECIFIC AIM 3 (Objective 3): Determine whether the reduction of α1-AR mediated signaling at the time of traumatic stress will prevent the subsequent development of increased alcohol abuse and PTSD,** *informing whether prophylactic prazosin treatment is likely to decrease vulnerability to PTSD and alcohol use disorders* **(TASK 6).**

STATUS: This work includes pharmacologic reduction of noradrenergic signaling at the time of traumatic stress to determine whether this treatment blocks subsequent development of a rat PTSD-like behavioral and acoustic startle profile, as well as increased subsequent IAA alcohol intake. As discussed in the years 2 and 3 Annual Reports, we expanded this study to evaluate not only prazosin but also prazosin+propranolol treatments, due to recent results in some of our unrelated studies (funded by a separate NIH grant). Results of trials with 141 male Wistar rats are described here. The initial results were included in the Year 3 progress report. A new post-doctoral fellow (Rebecca Hendrickson, MD, PhD) has now begun working in the lab and is helping to write up these results for publication, so final study data have been retained in the current report.

The rats received a single traumatic stress (TS, 10 sec inescapable footshock) or nonstress (NS, 10 sec exposure to shock environment, but without administration of shock) followed by weekly contextual reminders (R) of the TS or NS, as described in detail in the original proposal. Either prazosin (1.5 mg/kg), prazosin (1.5 mg/kg) + propranolol (5 mg/kg), or vehicle alone were administered by intraperitoneal (IP) injection 30-45 minutes before the TS or NS and again at 2 hours after the TS or NS. After 4-5 weekly R followed by 3-4 additional weeks of behavioral testing, the rats were allowed to voluntarily drink alcohol in an IAA model (20% ethanol *vs* water 2-bottle choice access for 24 hours/day on 3 non-consecutive days/week, as discussed in detail in the original grant proposal) for a total of 12 IAA trials (i.e., 3 IAA trials/week for 4 weeks).

Alcohol intake was determined in the first 1 hour as well as in all 24 hours of each IAA session. The results for the first hour of drinking (thought to reflect motivation to drink alcohol for its acute pharmacologic effects, rather than drinking for caloric content and other factors affecting 24 h drinking) and for all 24 hours of each IAA session are shown in **Fig. 9**. Alcohol intake in the first hour as well as in all 24 hours of each IAA trial increased gradually but irregularly in the 12 successive IAA trials ($p<0.001$ by 2-way ANOVA with repeated measures on IAA trial), consistent with the gradual increase in alcohol drinking previously reported for the IAA model. A relatively consistent treatment response pattern in the first 6 IAA sessions (i.e., the first 2 weeks, when alcohol intake was progressively increasing) was qualitatively different from the consistently elevated alcohol intake and consistent response pattern in IAA 7-14 (i.e., weeks 3-4). Consequently, subsequent analyses were then conducted after averaging data for each rat over sessions 1-6 and 7-14. **Fig. 10** illustrates suppression of IAA alcohol intake and alcohol preference by prazosin (PRZ) or prazosin+propranolol (PRZ+PRO) treatment only at the time of the single TS/NS exposure 8-12 weeks earlier.

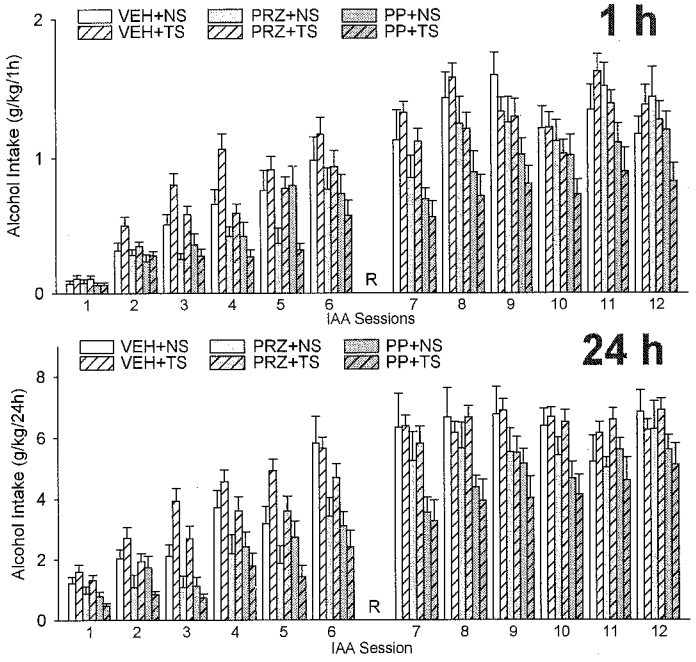

FIG. 9

Fig 10, Row 1: Alcohol intake during the first hour of each IAA, averaged across IAA trials 1-6 (LEFT PANEL) and 7-12 (RIGHT PANEL). In IAA trials 1-6 (LEFT PANEL), 1 h alcohol intake in rats that had received non-stress treatment (NS) 8 weeks prior to initiation of IAA was not significantly different among rats that had received VEH *vs* PRZ vs PRZ+PRO treatment at the time of the NS treatment. TS treatment 8 weeks prior to initiation of IAA increased 1 h alcohol intake ($p<0.05$ *vs* NS) in rats that had been treated with VEH or PRZ at the time of the TS exposure. The 1 h alcohol intake in rats that had received TS was suppressed by PRZ treatment at the time of TS ($p<0.05$ *vs* VEH), and further suppressed by PRZ+PRO treatment ($p<0.01$ *vs* PRZ alone). In IAA trials 7-12 (RIGHT PANEL), average alcohol drinking during the first 1 h of each IAA was suppressed by PRZ+PRO ($p<0.001$ *vs* VEH, $p=0.01$ *vs*

PRZ) treatment at the time of the single TS/NS exposure, independent of TS/NS exposure. Each bar represents data from 15-35 rats.

A preliminary study demonstrated that PRO (5 mg/kg) at the time of TS did not suppress (*vs* VEH) either 1 h or 24 h alcohol intake during IAA initiated 8 weeks later (data not shown).

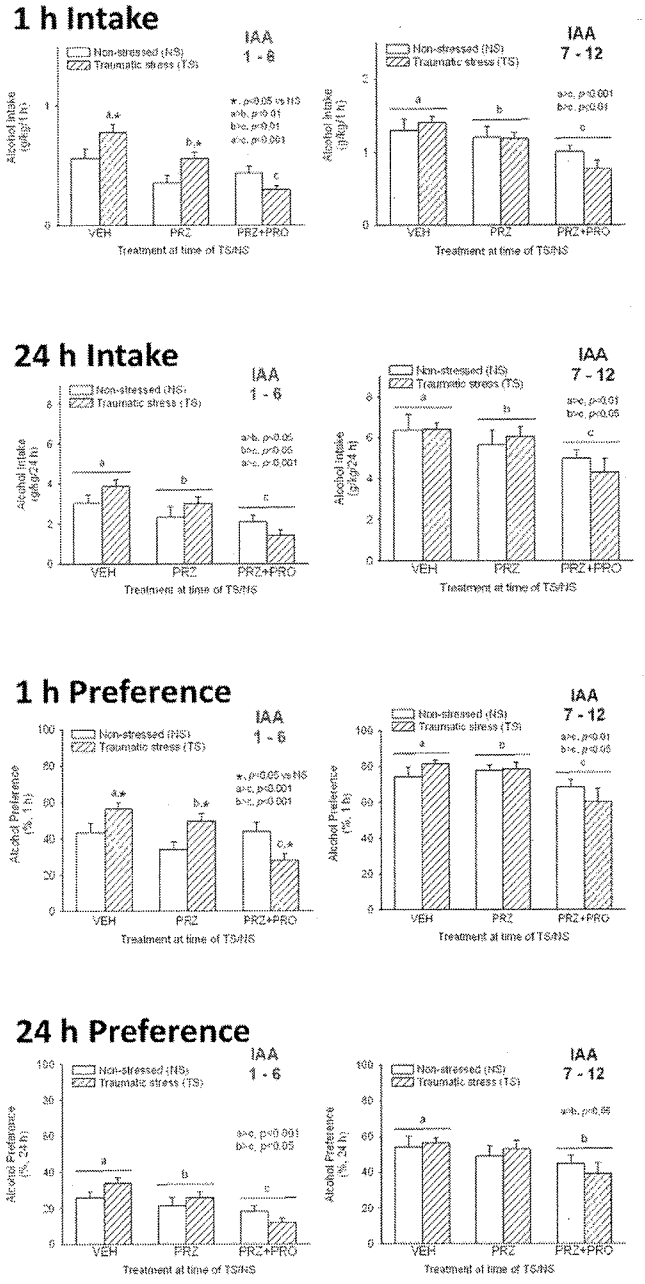

FIG. 10

Fig. 10, Row 2: Alcohol intake during the entire 24 hours of each IAA, averaged across IAA trials 1-6 (LEFT PANEL) and 7-12 (RIGHT PANEL). Average 24 h alcohol intake during IAA trials 1-6 (LEFT PANEL) was suppressed by PRZ treatment ($p<0.05$ *vs* VEH) - and further suppressed by PRZ+PRO treatment ($p<0.05$ *vs* PRZ, $p<0.001$ vs VEH) - at the time of the single TS/NS exposure 8 weeks earlier, independent of TS *vs* NS exposure. In IAA trials 7-12 (RIGHT PANEL), 24 h alcohol intake was suppressed by PRZ+PRO ($p<0.01$ *vs* VEH, $p<0.05$ *vs* PRZ) treatment at the time of the single TS/NS exposure, independent of TS *vs* NS exposure. Each bar represents data from 15-35 rats.

Fig. 10, Row 3: Alcohol preference during the first hour of each IAA, averaged across IAA trials 1-6 (LEFT PANEL) and 7-12 (RIGHT

PANEL). In the first 6 IAA trials (LEFT PANEL), 1 h alcohol preference in rats that had received non-stress treatment 8 weeks prior to initiation of IAA were not significantly different among rats that had received VEH *vs* PRZ *vs* PRZ+PRO treatment at the time of the NS treatment. TS treatment 8 weeks prior to initiatio n of IAA increased 1 h alcohol preference ($p<0.05$ *vs* NS) in rats that had been treated with VEH or PRZ at the time of the TS exposure; in contrast, TS at 8 weeks prior to IAA decreased alcohol preference ($p<0.05$ *vs* NS) in rats that had received PRZ+PRO at the time of the TS exposure. The 1 h alcohol preference in rats that had received TS was suppressed by PRZ+PRO treatment at the time of TS ($p<0.001$ *vs* VEH or PRZ). In IAA trials 7-12 (RIGHT PANEL), average 1 h alcohol preference in rats was suppressed by PRZ+PRO ($p<0.01$ *vs* VEH or PRZ) treatment at the time of the single TS/NS exposure, independent of prior TS/NS exposure. Each bar represents data from 15-35 rats.

A preliminary experiment demonstrated that PRO (5 mg/kg) at the time of TS did not suppress either 1 h or 24 h alcohol preference during IAA initiated 8 weeks later. A separate experiment demonstrated that PRZ+PRO treatment at the time of TS did not alter 1 h intake of 1% sucrose in the week following completion of IAA (data not shown).

Fig. 10, Row 4: Alcohol preference during the entire 24 hours of each IAA, averaged across IAA trials 1-6 (LEFT PANEL) and 7-12 (RIGHT PANEL). Average 24 h alcohol intake during IAA trials 1-6 (LEFT PANEL) was suppressed by PRZ+PRO treatment ($p<0.001$ *vs* VEH, $p<0.05$ *vs* PRZ), independent of TS *vs* NS exposure. In IAA trials 7-12 (RIGHT PANEL), 24 h alcohol intake was suppressed by PRZ+PRO ($p<0.05$ *vs* VEH) treatment at the time of the single TS/NS exposure, independent of TS/NS exposure. Each bar represents data from 15-35 rats.

These results suggest that a single traumatic stress followed by weekly contextual reminders of the stress can increase voluntary alcohol drinking by male Wistar rats ≥ 8 weeks after the traumatic stress.

Treatment with the α1-adrenergic antagonist prazosin (PRZ) or – especially – combination treatment with both PRZ and the β-adrenergic antagonist propranolol (PRO) to reduce noradrenergic signaling at the time of the traumatic stress can decrease or prevent this later development of increased alcohol drinking, even though the drugs were administered only at the time of the traumatic stress (i.e., 30 minutes before and again 2 h after the stress) whereas the increased alcohol drinking was expressed ≥ 8 weeks later. These results suggest that PRZ or PRZ+PRO (or other treatments that decrease noradrenergic signaling) at the time of traumatic stress could potentially provide prophylaxis for alcohol use disorders that commonly accompany development of PTSD. In contrast, a preliminary study demonstrated that PRO alone did not suppress the TS-induced IAA alcohol drinking.

The doses and times of PRZ or PRZ+PRO administration before traumatic stress in this study were the same as the doses and times that we previously demonstrated to acutely decrease alcohol drinking in rats without producing sedating or motor effects.

Trauma memory testing before the IAA was initiated, at 8 weeks after the single traumatic shock (TS), confirmed that the TS + reminders (R) rats remembered the single TS and that the context of the TS remained aversive (expressed as increased avoidance behavior and increased defecation during R, each $p<0.001$ relative to NS, results not shown). These results suggest that PRZ treatment at the time of TS can decrease subsequent development of increased voluntary alcohol drinking 8-12 weeks after TS, that this response to PRZ is markedly enhanced by co-treatment with PRO, and that these responses are probably not mediated by decreasing the perceived aversiveness of the TS or by decreasing memory of the TS.

PRZ or [PRZ+PRO] administration at the time of TS potentially may provide effective preventive treatment for TS-induced development of alcohol abuse and – perhaps – PTSD or other correlates of PTSD. Further investigations are warranted to address effects of treatments at other time points relative to TS and R, resolution of responses by TS-sensitive vs -resilient subjects, and mechanisms to identify potentially clinically-effective interventions.

We are also analyzing the extensive acoustic startle and behavioral test results as discussed in the original proposal, addressing potential correlations and predictive validity of characterizations before and after the rat PTSD model in determining development of a PTSD-like condition and/or increased alcohol intake. This overall Task is thus still approximately 75% complete.

- **CHANGES, PROBLEMS, DELAYS AND PLANS TO RESOLVE THEM**

There are no significant changes in objectives and scope.

As discussed in previous reports, a change from the original proposal was the incorporation of osmotic minipumps for long-term drug administrations. However, unanticipated problems with this method subsequently required that this method be abandoned and the experiment for Task 2 was re-done using the originally proposed method of repetitive oral administration and also, as discussed in the Year 3 report, administering the alpha-1 adrenergic receptor, doxazosin, rather than prazosin. As discussed in this report, this approach was successful, demonstrating that doxazosin treatment, decreasing the development of IAA alcohol drinking. However, suggestive evidence that this suppression of alcohol drinking by moderate dose doxazosin treatment was dependent upon pre-treatment acoustic startle response amplitude was not clear, so we will further evaluate this relationship by investigating an additional cohort of rats.

The order of the studies was changed in the Year 1 progress report and in the SOW revised at that time; at present all studies are complete or in progress, although it is now necessary to re-do some experiments to address problems, as discussed in this progress report.

As also previously noted in the Years 1 and 2 reports, it took longer than anticipated to recruit, hire (6 months after the award notice), process and train one new staff member (Shelby Johanson) in the first year, introducing delays at the start of the project. In Year 2, my long-time Laboratory Manager/Research Scientist, Carrie Kincaid, left my lab for a higher paying position as Research Manager for an extensive clinical research program at this VA medical center. After having her working with me for 10 years, this was of course highly disruptive. We then recruited, hired, processed and trained her replacement, Jennifer Burns, but she left the lab after 6 months when she received a fellowship to enter a neuroscience PhD program in Pittsburgh. Consequently, in the previous year we again hired, processed and trained her replacement, Kristen Baumann, who has proven to be an effective addition to the lab. Nonetheless, these repetitive personnel changes and the time that it has taken to get the new personnel up to speed and operating efficiently has caused problems and delayed progress, so much so that we now have entered a second 1-year no-cost extension period. We anticipate that this extension will allow effective completion of all studies, including sufficient time for appropriate thorough analysis, presentation and publication of results. One additional change that will now greatly facilitate this process has been the recent addition of a post-doctoral fellow, Rebecca Hendrickson MD PhD, to my lab.

- **SUMMARY DISCUSSION**

The first year was used for personnel recruitment and training, implementation of all necessary methodologies, completion of the first study (which is key to interpreting all subsequent studies), and initiating subsequent studies - each of which is an individually long-term study (ranging from months to greater than a half-year for each of multiple temporally-overlapping cohorts of subjects within each study). In the second year another large key study progressed well and initial results were enthusiastically received when presented at the annual meeting of the International Society of PsychoNeuroEndocrinology, a meeting that was focused on effects of stress on the brain. The final cohorts of animals have now completed all trials in this study and analyses of interactions of PTSD-like responses with alcohol drinking are underway. Some results from this final complete complement of animals were presented at the 2016 Annual Meeting of the Society for Research on Alcoholism and are currently in preparation for publication. In the now-completed 4th year we have made additional progress toward completing all proposed investigations. We are now wrapping up these studies, using additional cohorts of rats where needed. As these studies each are completed, we shift more to writing the results up for publication. With the additional time

of the additional year of no-cost extension, we anticipate effective and thorough completion of these important and high impact studies

4. KEY RESEARCH ACCOMPLISHMENTS

- The key new accomplishment in Year 4 is our demonstration that the effect of acute pharmacologic reduction of noradrenergic signaling on increased voluntary alcohol drinking is dependent on the individual rat's history of previous stresses as well as on the individual rat's previous history of repetitive high dose alcohol drinking and/or the associated development of compulsive alcohol drinking. This finding is important because it informs clinical trials in the path to implementing prazosin or other noradrenergic treatments to decrease alcohol abuse, since there has been large individual variability in responses to prazosin and doxazosin. We addressed this important issue in a national symposium for which the PI was co-organizer, presented at the 2017 annual meeting of the Research Society on Alcoholism and featuring basic science and clinical investigators interested in developing new treatments for both PTSD and alcohol abuse.
- Another key accomplishment in Year 4 is based on completing a thorough analysis of experimental results demonstrating that pharmacologic reduction of noradrenergic signaling at the time of a single traumatic stress prevents subsequent development of increased alcohol drinking long after the traumatic stress and long after the brief pharmacotherapy at the time of the trauma. This finding was important because it reveals a potential prophylactic approach in preventing effects of traumatic stress on subsequent alcohol drinking. Perhaps even more important, it also reveals additional potential clinical interventions that now can be readily tested with the model that we have developed, such as prevention of PTSD responses by suppressing noradrenergic signaling at other time points (e.g., in the time period immediately following a traumatically stressful experience to block development of PTSD or sequelae, or at the time of reminders to facilitate extinction therapy). Analyses of this study are now complete, facilitated by the recent addition of a post-doctoral fellow (Dr. Rebecca Hendrickson) who has also now started preparing a corresponding manuscript.
- Additional new findings in Year 4 are key to the successful completion of the remaining studies, even though they have introduced additional delays. First, demonstration that repetitive oral administration of doxazosin - which has a longer half-life than prazosin (allowing daily administration in a piece of sweetened gelatin containing the doxazosin that the rats readily consume)

effectively decreases development of increased alcohol drinking. Second, administration of traumatic stress at 1 week after removal of liquid diet containing alcohol, a model of previous alcohol abuse effects on response to traumatic stress, was unexpectedly found to block subsequent development of a PTSD-like syndrome (compared to TS administered without prior chronic alcohol consumption), similar to the previous (original) study with TS administered only 5 hours after chronic consumption of alcohol. This requires that an additional trial be conducted with rats receiving more protracted chronic alcohol consumption, with confirmation that the rats are clearly experiencing marked alcohol withdrawal at the time of a TS.

5. **CONCLUSION**:

The key results previously reported for Year 1 were consistent with the hypothesis that is central to all other studies in this research project, i.e. that hyper-responsiveness characteristic of PTSD, alcohol withdrawal/abstinence, and increased noradrenergic activation contributes to development of increased alcohol drinking. These results provided the conceptual basis for a potential approach to prospectively identifying individuals – including individuals with PTSD - at increased risk for future alcohol use disorders, thus allowing development and implementation of potential preventive interventions.

The key result from a large experiment primarily conducted in Years 2 and 3 now provides evidence for a promising potential preventive intervention. We have previously demonstrated that a single episode of traumatic stress in our rat PTSD model can produce sustained marked increases in hyper-responsiveness reflected in acoustic startle response, which is noradrenergic activation dependent; this subsequent key result, i.e., that reduction of noradrenergic signaling at the time of single traumatic stress prevents subsequent development of increased alcohol drinking long after the traumatic stress, demonstrates a potential pharmacologic intervention for preventing at least the subsequently increased alcohol drinking following a traumatic stress. With the addition of a post-doctoral Fellow to the lab, we are also evaluating what other aspects of PTSD-like rat behavior also respond to this pharmacologic intervention. The new key finding then also suggests subsequent related questions, such as "would treatment only immediately after a trauma also be effective?", or "would treatment only at the time of each contextual reminder also be effective?", and "what mechanisms are involved?" Addressing these subsequent questions would inform potentially effective pharmacotherapy in cases where traumatic stress had already recently occurred, or perhaps as an adjunct to subsequent PTSD psychotherapy. Together, these two initial key results also provide the conceptual bases for potential prospective identification of individuals – including individuals with PTSD – at increased risk for future alcohol use disorders, and then

potentially applying preventive and – possibly - therapeutic pharmacologic intervention.

An additional key result in Year 3 initially revealed that the response to reduction of noradrenergic signaling depended on previous history of stress exposure. Rats that had been exposed to traumatic stress and subsequent contextual reminders in the rat PTSD model subsequently exhibited increased voluntary IAA alcohol drinking, and administration of prazosin or prazosin+propranolol acutely suppressed this drinking. However, rats that had not experienced traumatic stress did not exhibit such elevated IAA drinking and this voluntary alcohol drinking was not significantly suppressed by prazosin or prazosin+propranolol. We have now (in Year 4) repeated this important experiment, with the same results. Together, these results inform - and provide a pre-clinical model for further investigating – the interpretation of variable responses to prazosin that have been reported in clinical investigations.

This investigation of variable responses to suppression of noradrenergic signaling was advanced further in Year 4 with the demonstration that rats with a long history of intermittent high-dose alcohol drinking that led to compulsive alcohol drinking responded to acute doxazosin treatment by decreasing alcohol intake, whereas rats with the same duration of continuous access to alcohol conversely increased alcohol intake in response to doxazosin. The result is consistent with recent evidence that alcoholic patients with a dense family history of alcoholism, who also exhibit compulsive alcohol drinking (e.g., they maintain high alcohol intake even in a clinical trial context) decrease alcohol drinking in response to doxazosin, whereas alcoholic patients without a dense family history of alcoholism and without evidence of compulsive alcohol drinking (e.g., they drink little alcohol in the clinical trial setting) conversely increased alcohol intake in response to doxazosin. The PI co-organized and presented in a symposium at the recent Research Society on Alcoholism meeting, focused on this variability in responses to prazosin and prospective prediction/evaluation of who is most likely to respond. These studies are already having a significant impact on the direction of research and clinical implementation related to prazosin and doxazosin treatments

The remaining in progress studies will further develop a model that will be useful for current and future investigations of neurobiological mechanisms mediating initiation and development of excessive drinking, mechanisms mediating co-morbidity of alcohol use disorders and PTSD, and additional potential treatments of both.

In this final year of work, manuscripts from the unpublished studies will also be prepared.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

a. New manuscripts in Year 4

Lay press: Nothing to report

Peer-Reviewed Scientific Journals:

Rasmussen DD, Kincaid CL, Froehlich JC 2017 Prazosin prevents increased anxiety behavior that occurs in response to stress during alcohol deprivations. Alcohol Alcohol 52:5-11. doi:10.1093/alcalc/ag082. Epub 2016 Oct 26. PMCID: 5169035. This paper was generated from work supported by another grant but it supports and complements ongoing studies in this project and thus is included here.

No additional new papers to report in the 4th year; two are currently in preparation.

Invited Articles: Nothing to report

Published abstracts:

Rasmussen DD, Johanson SS, Burns JL, Kincaid CL. Reduction of α1- and β-adrenergic signaling at the time of traumatic stress reduces subsequent development of increased alcohol drinking in rats. Alcohol.Clin.Exp.Res. 40 (S1):198A, 2016.

Rasmussen DD. Variability in alcohol drinking responses to suppression of noradrenergic signaling in outbred rats. Alcohol.Clin.Exp.Res. 41 (S1):236, 2017.

b. Presentations in Year 4:

The PI presented work described in an appended published abstract to the 2016 Annual Meeting of the Research Society on Alcoholism: Rasmussen DD, Johanson SS, Burns JL, Kincaid CL. Reduction of α1- and β-adrenergic signaling at the time of traumatic stress reduces subsequent development of increased alcohol drinking in rats. Alcohol.Clin.Exp.Res. 40 (S1):198A, 2016. PMID not assigned.

The PI was Co-Organizer (together with Reuben Gonzales PhD) of a symposium presented at the 2017 annual meeting of the Research Society on Alcoholism, an international society: The Neglected Catecholamine: Noradrenergic Mechanisms in Regulation of Alcohol-Related Behaviors. Alcohol.Clin.Exp.Res. 41 (S1): 13A, 2017. PMID not assigned.

The PI presented a lecture at the 2017 annual meeting of the Research Society on Alcoholism work, described in an appended published abstract: Rasmussen DD. Variability in alcohol drinking responses to suppression of noradrenergic signaling in outbred rats. Alcohol.Clin.Exp.Res. 41 (S1):236, 2017. PMID not assigned.

7. **INVENTIONS, PATENTS AND LICENSES**: Nothing to report

8. **REPORTABLE OUTCOMES:** Our initial key finding in Year 1, that acoustic startle in alcohol-naïve rats is highly predictive of subsequent voluntary IAA alcohol drinking and preference, extends and complements the results of one of our previous studies demonstrating that pre-stress acoustic startle predicts development of rat PTSD-like further increased acoustic startle and plasma corticosterone response following a traumatic stress. The key finding from preliminary findings initially reported in Years 2-3 demonstrating that reduction of noradrenergic signaling at the time of a single traumatic stress prevents subsequent development of increased alcohol drinking long after the traumatic stress, extends and complements our Year 1 key finding by revealing a potential new pharmacotherapeutic approach for preventing at least the subsequently increased alcohol drinking following a traumatic stress. Together these results suggest that increased acoustic startle and associated increased anxiety - both of which are increased by noradrenergic activation - reflect underlying mechanisms that increase vulnerability to both PTSD and alcohol abuse. Together with our and others' previous results demonstrating that prazosin can decrease both voluntary alcohol intake and PTSD symptoms, these results strongly suggest that prazosin can be effective for both conditions and that an α1-adrenergic receptor-mediated mechanism is at least one component of the common underlying mechanism, and thus an especially appropriate target for both prophylactic and therapeutic interventions. However, another key finding in Year 3 further refined this interpretation by demonstrating that the voluntary alcohol drinking response to decreasing noradrenergic signaling appears to be dependent upon prior history of traumatic stress experience. This important result was confirmed in an additional study in Year 4. In Year 4 it was also demonstrated that rats that had developed "compulsive-like" drinking following prolonged repetitive high-dose alcohol drinking in an intermittent access model also decreased this alcohol drinking in response to doxazosin, whereas rats that received continuous access to alcohol over the same time period did not develop high levels of voluntary alcohol drinking and their alcohol drinking was conversely increased in response to doxazosin. Although this complicates interpretations of the overall results, it is consistent with the evidence that all clinical subjects do not respond equally to prazosin or doxazosin treatment, and that alcohol-dependent patients with a high density family history of alcoholism, exhibiting consistent drinking of high levels of alcohol (e.g., compulsive alcohol drinking), decrease their alcohol drinking in response to doxazosin; in contrast patients without high density family history of alcoholism voluntarily decrease their drinking in a clinical study setting and conversely exhibit increased alcohol drinking in response to doxazosin. Our studies provide an effective preclinical model for further resolving this variability in responses, prospectively identifying who is most likely to respond, and developing new treatments, or effective treatment combinations (such as prazosin or doxazosin administered together with

naltrexone, which we demonstrated in studies - supported by a separate NIH grant – to suppress alcohol drinking in female alcohol-preferring (P) rats even when prazosin alone did not). The remaining studies further investigate these interactions, facilitating most effective translation of prazosin treatment to clinical utility. All studies are now nearing completion and, although there have been setbacks due to unexpected personnel turnovers and to methodologic issues that have made it necessary to now repeat some of the experiments (in some cases, several times), these studies are nonetheless progressing well to successful completion. Our further development of the rat PTSD model, employing a single traumatic stress together with weekly brief contextual reminders of the stress will – together with the further characterization of PTSD-like responses in these studies – also provide a well-characterized experimental model for other labs investigating PTSD and alcohol abuse, alone or together. In addition, a) the findings, results and techniques of these studies are directly applicable to other investigations of the effects of stress or the evaluation of mechanisms contributing to voluntary alcohol and other drug abuse, b) the results of this investigation will facilitate translating prazosin (and doxazosin) treatment to clinical implementation in the treatment of PTSD and alcohol abuse, alone or together, and c) our results will ultimately improve overall understanding and effective treatment of alcoholism and PTSD, two conditions with profound negative social and economic impact.

9. **OTHER ACHIEVEMENTS:** A great deal of basic and clinical PTSD and related traumatic brain injury (TBI) research is based at the VA Puget Sound Health Care System (VAPSHCS) Mental Illness Research, Education and Clinical Center (MIRECC), providing ample ongoing collaborative opportunities. The PI works closely with a PTSD clinical investigator colleague, Dr. Murray Raskind, as well as alcohol clinical investigators (Drs. Andrew Saxon and Tracy Simpson) and a TBI clinical investigator, Dr. Elaine Peskind, within the VAPSHCS and MIRECC to facilitate translation of basic science findings in the current investigation to clinical testing and future clinical implementation as discussed in Section 8.

10. REFERENCES

Rasmussen DD, Johanson SS, Burns JL, Kincaid CL. Reduction of α1- and β-adrenergic signaling at the time of traumatic stress reduces subsequent development of increased alcohol drinking in rats. Alcohol.Clin.Exp.Res. 40 (S1):198A, 2016. PMID not assigned.

Gonzales R, Rasmussen D (organizers). The neglected catecholamine: noradrenergic mechanisms in regulation of alcohol-related behaviors. Rasmussen DD, Johanson SS, Burns JL, Kincaid CL. Reduction of α1-

and β-adrenergic signaling at the time of traumatic stress reduces subsequent development of increased alcohol drinking in rats. Alcohol.Clin.Exp.Res. 41 (S1):13A, 2017. PMID not assigned.

Rasmussen DD. Variability in alcohol drinking responses to suppression of noradrenergic signaling in outbred rats. Alcohol.Clin.Exp.Res. 41 (S1):236, 2017. PMID not assigned.

11. **APPENDICES:** One published abstract and one symposium listing containing the summary of the 2017 Research Society on Alcoholism symposium (for which the PI was Co-Organizer) as well as an abstract of the lecture presented by the PI in the symposium.

Alcohol. Clin. Exp. Res. 40(S1): 198A, 2016

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REDUCTION OF α1- AND β-ADRENERGIC SIGNALING AT THE TIME OF TRAUMATIC STRESS REDUCES SUBSEQUENT DEVELOPMENT OF INCREASED ALCOHOL DRINKING IN RATS

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We previously demonstrated that pre-stress acoustic startle response (ASR) predicts subsequent further increased ASR in a rat PTSD model in which a single traumatic stress (TS) is followed by repeated weekly contextual reminders (R) of the TS. We also demonstrated that ASR predicts subsequent development of increased voluntary alcohol drinking in rats. Since noradrenergic activation contributes to increased ASR, anxiety, alcohol drinking, and PTSD, we hypothesized that reduction of noradrenergic signaling at the time of TS would decrease the subsequent development of increased alcohol drinking that commonly accompanies development of PTSD and increased anxiety. Male Wistar rats received either TS (10 sec footshock) or no-shock (NS). After 4 weekly R, intermittent alcohol access (IAA; choice between water and 20% alcohol for 24 h/day on 3 non-consecutive days/week) was provided for 4 weeks. Combined IP administration of the α1-adrenergic antagonist, prazosin (PRZ, 1.5 mg/kg), and the β-adrenergic antagonist, propranolol (PRO, 5 mg/kg), at the time of the single 10 sec TS decreased average alcohol intake by 46% in the first hour of each IAA, 8–12 weeks after the TS ($p < 0.001$, relative to treatment with vehicle). Administration of PRZ (1.5 mg/kg) alone at the time of the single TS also decreased average alcohol intake ($p < 0.001$, relative to vehicle), but only by 32%, which was less than the suppression by [PRZ+PRO] ($p < 0.01$). Alcohol preference was likewise suppressed more by [PRZ+PRO] than by PRZ alone ($p < 0.01$). Trauma memory testing before the IAA was initiated at 8 weeks after the single TS confirmed that the TS+R rats remembered the single TS and that the context of the TS remained aversive (expressed as increased avoidance behavior and increased defecation during R, each $p < 0.01$ relative to NS). These results suggest that PRZ treatment at the time of a TS can decrease subsequent development of increased voluntary alcohol drinking 8–12 weeks after TS, that this response to PRZ is enhanced by co-treatment with PRO, and that these responses are not mediated by decreasing the perceived aversiveness of the TS or by decreasing memory of the TS. PRZ or [PRZ+PRO] administration at the time of TS potentially may provide effective preventive treatment for TS-induced development of alcohol abuse and – perhaps – PTSD or other correlates of PTSD. Further investigations are addressing effects of treatments at other time points relative to TS and R, resolution of responses by TS-sensitive versus -resilient subjects, and mechanisms. Supported by VA Puget Sound Health Care System, Seattle, WA and by US Army Medical Research CDMRP W81XWH-13-1-0126.

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IMPACT OF CHRONIC ETHANOL SELF-ADMINISTRATION ON KAPPA OPIOID RECEPTOR REGULATION OF DOPAMINE SIGNALING IN NONHUMAN PRIMATES

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Although alcoholism is one of the most prevalent disorders in the United States, with over 18 million individuals meeting the criteria for an alcohol use disorder, the exact neurobiological bases of this condition remain obscure. Recently, it has been demonstrated that kappa-opioid receptor (KOR) signaling in the striatum plays a critical role in the increased reinforcing efficacy of ethanol following ethanol vapor exposure in rodent models. Here we examined the effects of chronic voluntary ethanol self-administration in macaques on dopamine neurotransmission and the ability of KORs to regulate dopamine release in the nucleus accumbens core. Three cohorts of nonhuman primates were given free access to 4% ethanol (w/v) for 22 h/day. These cohorts were composed of male cynomolgus, female rhesus or male rhesus macaques, and were given access to ethanol for 6, 12, or 18 months, respectively. Ex vivo fast-scan cyclic voltammetry was then conducted the nucleus accumbens core to determine dopamine signaling kinetics as well as the ability of U50,488 (KOR agonist) to inhibit dopamine release. We found that chronic ethanol drinking increased dopamine uptake rates, which could have implications for reductions in basal dopamine tone in vivo during ethanol withdrawal. Further, across sex, strain and exposure length, ethanol use augmented the ability of KORs to inhibit dopamine release, demonstrating that ethanol-induced increases in KOR sensitivity are widespread and independent of other factors. Finally, KOR sensitivity was positively correlated with lifetime ethanol intake, suggesting that changes in KOR regulation of dopamine release may be a determinant of aberrant drinking behaviors. Nonhuman primate models of ethanol abuse represent a highly translational avenue for identifying molecular targets for pharmacotherapeutic compounds, and here we show, for the first time, that voluntary ethanol self-administration has a unique effect on KOR sensitivity and regulation of dopamine release directly at the dopamine terminal that was positively correlated with drinking behavior. Together, these data provide novel insight into ethanol-induced dysregulation of opioid signaling and suggest that dopaminergic dysfunction may be mediating increases in voluntary drinking. Importantly, KOR antagonists may be efficacious in reducing drinking behaviors in alcoholics.

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TAAR1 ACTIVATION PREFERENTIALLY DECREASES PHASIC DOPAMINE RELEASE AND DECLINES ETHANOL SEEKING BEHAVIOR IN RATS

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Recent studies suggest that trace amine-associated receptor 1 (TAAR1) may represent a novel therapeutic target for the treatment of several neuropsychiatric disorders, including addiction. However, the mechanisms involved in the psychotropic actions of TAAR1 agonists are still unclear. The present study explored the effects of a partial TAAR1 agonist, RO5263397, on presynaptic dopamine (DA) transmission, using optogenetics combined with real-time DA detection. We also assessed the effect of this compound on operant ethanol drinking behavior. We applied a new viral technology to restrict the expression of ChR2 to DA cells in the VTA of Long Evans rats, driving ChR2-EYFP expression via a tyrosine hydroxylase promoter. The viral construct was microinjected into the VTA of rats ($n = 5$) and DA release was measured by fast-scan cyclic voltammetry in anesthetized rats. Importantly, the level of ChR2 expression was sufficient to allow us to optogenetically mimic tonic and phasic patterns of accumbal DA release. The experiments indicated a significant effect of RO5263397 (10 mg/kg, i.p.) on the amplitude of the optogenetically-induced DA signal ($p < 0.0001$). Notably, the compound differentially affected phasic and tonic patterns of DA release. Thus, the drug decreased phasic DA release more powerfully than tonic DA efflux ($p < 0.05$). Since there are marked regional variations in DA dynamics between striatal and accumbal terminals, we explored local effects of RO5263397 on DA efflux in the nucleus accumbens core, shell and dorsal striatum. The study was performed on brain slice preparations, where a single pulse (4 ms) of electrical stimulation was applied to evoke DA transients. High concentrations of the compound significantly decreased DA release in the nucleus accumbens core ($p < 0.05$ for 50 μM and $p < 0.0001$ for 100 μM) and dorsal striatum ($p < 0.0001$ for 100 μM). There was a significant difference in the effects of the compound at 50 and 100 μM between regions ($p < 0.05$). Specifically, electrically-evoked DA release in the shell region was less responsive to RO5263397 application. These in vitro data suggest that RO5263397 at high concentrations can affect presynaptic DA receptors at the level of terminals (preferentially, in the nucleus accumbens core and dorsal striatum). Finally, the effects of the compound on ethanol drinking behaviors were studied using the operant self-administration procedure. The rats were trained to press a lever 30 times daily for 20 min access to 10% ethanol. After subjects displayed stable appetitive and consummatory behaviors (≈6 weeks), the effect of RO5263397 (5 or 10 mg/kg, i.p.) or saline were evaluated. Pretreatment with the TAAR1 agonist significantly suppressed lever press behaviors at both doses tested with a main effect of treatment ($p < 0.001$). Importantly, when access to ethanol was provided, no significant changes in consummatory measures (e.g. number of licks, ethanol intake) were observed. In conclusion, these results suggest that the TAAR1 agonist tested selectively suppresses ethanol seeking behaviors, presumably through the inhibition of phasic DA release in the nucleus accumbens. This proposes the fascinating possibility that TAAR1 agonists may be considered as promising candidates for the treatment of alcohol addiction.

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DHM EFFECTS ON ETHANOL-INDUCED DOPAMINE RELEASE IN THE RAT NUCLEUS ACCUMBENS

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Direct and indirect involvement of the inhibitory GABA-A and glycine receptors has previously been demonstrated to be of importance for the ability of ethanol (EtOH) to increase dopamine levels in the nucleus accumbens (nAc). In a series of studies, we suggested that EtOH primarily acts in the nAc, where it triggers a neuronal circuitry involving nAc glycine receptors as well as ventral tegmental nicotinic acetylcholine receptors, in order to elevate extracellular accumbal dopamine. We have also investigated a number of substances known to decrease EtOH intake in rodents and/or humans, alone or in combination with EtOH with regards to influence on nAc dopamine. In the present study we aimed to explore the influence of dihydromyricetin (DHM) alone and in combination with EtOH on extracellular levels of dopamine in the nAc, as measured by in vivo microdialysis in freely moving Wistar rats. Since EtOH previously was demonstrated to increase endogenous levels of the amino acid taurine, a ligand to both GABA-A and glycine receptors, and DHM previously was suggested to mediate its EtOH reducing effects via the GABA-A receptor we also measured taurine, glycine, serine and glutamate in the dialysate samples. After systemic administration of DHM we found a modest (approximately 20%) increase of nAc dopamine whereas no alteration of any of the measured amino acids was detected. In rats pretreated with DHM a systemic injection of EtOH (2.5 g/kg i.p. 20 min after DHM) failed to produce any further dopamine elevation. In the present study DHM, a substance with potential interest for treating human alcohol use disorders, was found to display a similar pattern with regards to nAc dopamine as previously found for several other investigated compounds (acamprosate, varenicline and the glycine uptake inhibitor Org-25935). Thus, since yet another compound with affinity for inhibitory receptors in the nAc was demonstrated to produce a modest dopamine increase and prevent EtOH from further increasing extracellular dopamine levels we suggest that this drug profile is beneficial in the purpose of controlling excessive EtOH intake in humans.

PUTTING IMPLICIT ALCOHOL COGNITIONS TO THE TEST IN PERSONALITY, CLINICAL, AND DEVELOPMENTAL MODELS OF ALCOHOL RISK
ORGANIZER/CHAIR: ROISIN O'CONNOR

RATIONALE AND CONTENT: Psychosocial cognitive theories have long pointed to the role of memory associations in predicting risk for alcohol misuse. For example, if one learns that alcohol enhances positive mood or takes the edge off, then drinking to regulate internal states is likely. Through extensive empirical tests and revisions to and integration of theories, the nuances of problematic drinking consistently emerged and presented a conceptual challenge. While these models accounted for the effects of positive alcohol memory associations (*alcohol makes me feel good*) on alcohol use, they did not address the negative expectancies (*alcohol is ruining my marriage*) that risky drinkers likely hold, and which are important determinants of drinking. Delayed discounting theories offer some resolution, taking into account the relative value of both immediate positive effects (reward) and delayed negative effects (MacKillop et al., 2011). However, the mechanistic cognitive process is not well accounted for in these theories. Dual cognitive process models offer a unifying framework (Chaiken & Trope, 1999), suggesting that two modes of cognitive processing influence alcohol use. These are: (1) a reflective, deliberate process that engages top down reasoning to arrive at a thoughtful decision; (2) a reflexive process that is automatically activated by internal or environmental cues. The former is often referred to as a self-regulatory or controlled process and is typically measured using explicit self-reports. The second is often referred to as an impulsive, implicit, or automatic process and is typically measured using computer-administered reaction time assessments [Implicit Association Test (Greenwald et al., 1995) and its variants]. These models suggest that alcohol use is determined by the interplay of these processes (Wiers et al., 2007, 2010). The self-regulatory process, which permits thoughtful decisions about the cons of drinking, can be diminished by state affect, extensive alcohol abuse, and environmental triggers. With inhibited control, implicit alcohol cognitions take precedence. Thus, implicit cognitions that favour alcohol use are thought to be pivotal to alcohol risk. The goal of this symposium is to present recent findings that put this theory to the test. The link between implicit alcohol cognitions and drinking behavior has been well studied. Some of this work comes from the labs of the presenters and discussant for this symposium. This work is important and has supported the distinction of explicit and implicit alcohol cognitions. However, it is time to challenge these models further, putting them to the test by integrating them within personality, clinical, and developmental models of alcohol misuse risk. Our driving question is whether implicit alcohol cognitions perform as expected, thereby providing clarity to risk models. We will respond to this question by presenting results from experimental and prospective studies. In the first set of talks, Dr. Read (Talk 1) and Dr. O'Connor (Talk 2) will present work from experimental studies that used cue exposure and mood manipulations to push implicit cognitions around in the lab. In the second set of talks, Dr. Colder (Talk 3) and Dr. Keough (Talk 4) will present findings from two formative developmental periods, these include early-to-late adolescence and the transition out of university. Our discussant, Dr. Wiers, who has made substantial contributions to the dual process and alcohol use literature will help to pull these studies together, pointing towards next steps in theoretical and etiological alcohol use model development.

INTRODUCTION - ROISIN M O'CONNOR

USING THE IAT TO EXAMINE ALCOHOL COGNITIONS IN PTSD AND TRAUMA CUE EXPOSURE - JENNIFER READ - Dr. Read will present a lab-based study that tests the mechanistic role of implicit cognitions in PTSD risk for alcohol misuse. This work investigates trauma cue effects on implicit alcohol cognitions within clinical PTSD and non-clinical samples. The results suggest that emotionally laden cues bring positive alcohol (implicit) associations to the forefront, thus helping to explain how trauma may trigger alcohol use.

SOCIAL ANXIETY RELATED SHIFTS IN IMPLICIT COGNITION FOLLOWING ALCOHOL INITIATION IN THE LAB - ROISIN O'CONNOR - Dr. O'Connor will present a study examining how social anxiety-related implicit alcohol cognitions unfold from pre-to-post alcohol initiation in the lab. Results suggest that a priming dose of alcohol promotes activation of tension reduction alcohol cognitions for those high in social anxiety. This work points to in-the-moment shifts in implicit cognition that might help to explain social anxiety risk for alcohol misuse.

SENSITIVITY TO REWARD AND PUNISHMENT SHAPE IMPLICIT ALCOHOL ATTITUDES IN ADOLESCENCE - CRAIG COLDER - Dr. Colder will present a prospective study that examines the effect of individual differences on the shaping of implicit alcohol cognitions during the transition from early-to-late adolescence. Results of this seven-year study align with theory, in that youth motivated by reward (who were less responsive to punishment) showed a weakening of negative implicit alcohol cognitions across time.

EXAMINING IMPULSIVE AND SELF-REGULATORY COGNITIVE PROCESSES AS PREDICTORS OF MATURING OUT OF ALCOHOL MISUSE AMONG YOUNG ADULTS - MATTHEW KEOUGH - Dr. Keough will present a study that examines the effect of implicit and self-regulatory cognitive processes on the maturing out (of problematic drinking) phenomenon we see post-university. Results of this one-year prospective study suggest that while negative implicit alcohol cognitions coincide with maturing out, self-regulatory processes may overcome this bias and promote risk. This interplay of cognitive processes fits with theory.

DISCUSSANT / QUESTION MODERATOR - REINOUT WIERS - Dr. Wiers will frame the discussion around the application of contemporary dual process models of addiction within developmental and clinical contexts. He will link these studies to extant empirical evidence and highlight next steps for this line of query.

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THE NEGLECTED CATECHOLAMINE: NORADRENERGIC MECHANISMS IN REGULATION OF ALCOHOL-RELATED BEHAVIORS
ORGANIZER/CHAIR: RUEBEN GONZALES
ORGANIZER: DENNIS RASMUSSEN

RATIONALE AND CONTENT: Little attention has been given over the years to the potential role of norepinephrine in the mechanism of action of alcohol, especially compared to dopamine, a related neurotransmitter as well as the biosynthetic precursor of norepinephrine in neurons. However, recent work has sparked new interest in norepinephrine systems as a target for alcohol's central actions. Norepinephrine is involved in cognition, attention, and stress responses, all of which may contribute to behavioral effects of acute and chronic ethanol. This symposium brings together basic and clinical researchers to present recent findings that support the notion that norepinephrine pathways in the brain are altered by ethanol and that adrenergic drugs may be useful for treatment of alcohol use disorders.

Research Society on Alcoholism Annual Mtg 2017, Symposium + Oral presentation

INTRODUCTION - LORENZO LEGGIO

CHRONIC STRESS AND ETHANOL-MEDIATED ALTERATIONS IN NOREPINEPRHINE TRANSMISSION IN MESOLIMBIC CIRCUITRY- ANUSHREE KARKHANIS - Several pre-clinical studies have shown that rodents exposed to ethanol chronically lead to dependence and escalated intake of ethanol. Similarly, rodents exposed to chronic stress show significantly greater ethanol intake compared to their unstressed controls, thus showing a phenotype of increased vulnerability to develop alcohol use disorder. It is known that norepinephrine is modulated by exposure to stress and mediates anxiety-like behavior. The effects of chronic stress and chronic ethanol exposure on norepinephrine transmission in the basolateral amygdala (BLA) and the nucleus accumbens (NAc) were studied. Chronic stress results in ethanol (1 g/kg)-induced norepinephrine release compared to a lack of ethanol effect in controls in the NAc. Furthermore, an augmented norepinephrine response to a higher dose of ethanol (2 g/kg) was observed in chronic stress exposed ethanol naïve rats in both regions. Animals exposed to chronic ethanol vapor showed greater norepinephrine in the BLA at baseline compared to air-exposed controls. A pharmacological stressor resulted in an enhanced norepinephrine response in ethanol-exposed animals. These results imply that the norepinephrine system is primed and stimulation of this system results in a sensitized response in animals exposed to chronic stress and/or ethanol.

NOREPINEPHRINE RESPONSE TO ACUTE ADMINISTRATION OF ETHANOL AND AFTER ETHANOL SELF-ADMINISTRATION IN THE MEDIAL PREFRONTAL CORTEX - ASHLEY VENA - The pharmacological effects of ethanol on extracellular norepinephrine concentrations in the medial prefrontal cortex following two different routes of ethanol administration were determined. An acute intravenous infusion of ethanol stimulated dialysate norepinephrine concentrations to 77% over baseline, while saline infusion did not enhance the response. Microdialysis was also performed in rats during an operant self-administration session. Rats experienced about one week of self-administration of a sweetened ethanol solution (10S10E) or a sucrose solution (10S). A separate control group did not receive any drinking solutions in the operant chambers. Basal norepinephrine concentrations were significantly different among the three groups: 0.40±0.06 nM in the 10S10E group, 0.55±0.01 nM in the handling group, and 0.62±0.07 nM in the 10S group. During the operant session, a transient spike in norepinephrine was observed during the transfer from the home cage to the operant chamber that lasted for the duration of the wait period in all three groups. The results show that ethanol exerts direct pharmacological actions on central noradrenergic neurons. Furthermore, limited voluntary ethanol consumption appears to be sufficient to alter tonic norepinephrine signaling in the medial prefrontal cortex.

VARIABILITY IN ALCOHOL DRINKING RESPONSES TO SUPPRESSION OF NORADRENERGIC SIGNALING IN OUTBRED RATS - DENNIS RASMUSSEN - Voluntary alcohol drinking in selectively-bred alcohol-preferring (P) rats is suppressed by administration of the α1 adrenergic antagonists, prazosin or doxazosin. This suppression by prazosin is consistent among diverse experimental conditions and is increased when combined with the β-adrenergic antagonist, propranolol. However, selectively-bred P rats do not exhibit the genetic and behavioral diversity characteristic of outbred rats or humans, and responses to prazosin and doxazosin have been demonstrated to vary among individuals within clinical studies. Dr. Rasmussen will examine responses to prazosin in outbred Wistar rats that likewise exhibit variable alcohol drinking responses that are dependent upon gender, individual behavioral characteristics, and alcohol exposure history. He will also present results from a study with Wistar rats in a model of PTSD combined with intermittent alcohol access, in which suppression of alcohol drinking by either prazosin or prazosin+propranolol ($p<0.001$ for each) was dependent upon history of traumatic stress. The variability in voluntary alcohol drinking responses in outbred rat models may inform clinical studies with the goal of prospectively identifying which patient subpopulations are most likely to decrease alcohol drinking in response to suppression of noradrenergic signaling.

THE ALPHA-1 BLOCKADE BY DOXAZOSIN IN REGULATION OF ALCOHOL DRINKING IN ALCOHOL-DEPENDENT PATIENTS - CAROLINA HAASS-KOFFLER - Evidence suggests that α1 blocker prazosin reduces alcohol drinking in alcohol-dependent (AD) individuals, and pre-treatment blood pressure (BP) predicts therapeutic response in PTSD patients. The α1 blocker doxazosin has pharmacokinetic advantages over prazosin, but has never been studied for AD. Dr. Haass-Koffler will present the data from a double-blind placebo controlled randomized clinical trial (RCT) conducted in AD individuals seeking outpatient treatment. Previously reported RCT data shows that doxazosin reduced drink per week [DPW, $p_{corrected}<0.001$, $d=1.30$] and heavy drinking days [HDD, $p_{corrected}<0.001$, $d=1.30$] in patients with *high* family history density of alcoholism (*FHDA*). New unpublished results now shows, with pre-treatment standing diastolic BP as moderator, there were significant BP x medication interaction for DPW [$p<0.01$, $d=.80$] and HDD [$p=0.05$, $d=1.11$]. *Post-hoc* analyses showed a significant medication effect in patients with *high* BP in reducing DPW and HDD. Taken together, these findings suggest that doxazosin may be effective selectively in AD patients with high *FHDA* and that *higher* standing diastolic BP at baseline. The results obtained from this RCT provide evidence for a personalized medicine approach on the use of α_1 blockade to treat AD patients.

DISCUSSANT / QUESTION MODERATOR - RAYE LITTEN

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NON-CODING RNAS IN ALCOHOLIC LIVER DISEASE: FROM BIOMARKERS TO TARGETED THERAPEUTICS
ORGANIZERS/CHAIRS: SUTHAT LIANGPUNSAKUL AND LI WANG
CHAIR: DALE HERELD

RATIONALE AND CONTENT: The new landscape of human transcriptome along with the identification of non-coding RNAs (ncRNAs) has uncovered their importance in the pathophysiology of human diseases. A non-coding RNA (ncRNA) is an RNA molecule that is not translated into a protein. Non-coding RNA genes include highly abundant and functionally important RNAs such as microRNAs and the long non-coding RNAs (lncRNAs). A microRNA (miRNA), a small non-coding RNA molecule containing about 22 nucleotides, functions in RNA silencing and post-transcriptional regulation of gene expression. lncRNAs are a subgroup of ncRNAs with approximately 200 nucleotides. lncRNAs are expressed at lower levels when compared to protein-coding genes. They are known to regulate gene transcription and involve in controlling metabolic processes. Metabolic derangements in fat cell metabolism and adipogenesis, commonly seen in excessive alcohol use subjects, are also under the regulation of lncRNAs. Recent studies have shown the importance of these ncRNAs in the pathogenesis of several liver diseases including alcoholic liver disease. The goal of this symposium is to summarize the roles of ncRNAs from bench, the mechanism and their function in liver diseases, to bedside, the clinical implications in patients with alcoholic liver disease.

INTRODUCTION - DALE HERELD